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The primary Synthesis of Proteids in Plants

BY W. M. KOZLOWSKI

Our knowledge concerning the primary products of carbon synthesis and the formation of carbohydrates in plants is in a degree exact,* but regarding the question of synthesis of proteids we are not in a position to give any plausible reasons for our suppositions. This is not strange, when we consider that the very product of this synthesis is imperfectly known and that until recently we have had no rational nor even empirical formula of proteids which we could assume with any degree of probability.

I believe that little worthy of note will be omitted if I divide into four classes the views set forth in the scientific literature upon the structure of proteids.

1. Hunt considered these as nitryls of sugar, derived from the latter and ammonia, a supposition which can hardly be reconciled with the recent results of physiological and chemical investigations.

2. Sachsse holds that they are anhydrides produced from asparagin and from fatty aldehydes.

3. According to Schützenberger they are composed of ureids, derivatives of carbamide.

4. Finally, Grimaux, in his definition of proteids as compounds which by addition of water are decomposed into carbonic

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*The author has shown in a Polish paper (Wezechswiat, 1893, Nos. 4 and 5) that the recent discoveries of E. Fischer make equally plausible three suppositions about the formation of carbohydrates from CO_2 and H_2O in plants; one of them is the well-known hypothesis of Bayer, the second was proposed by E. Fischer himself and the third by the author of the quoted paper.

[Issued February 8.]

acid, ammoniac and amidic acids, places them in the nearest vicinity with uric acid, which gives also by hydrolysis carbonic acid, ammoniac acid, glycocol.

The principal facts to be accounted for with these different hypotheses are the following:

1. The last product containing nitrogen in the metamorphosis of animals is carbamide or uric acid.

2. The compounds from which proteids are formed in plants, as will be shown later on, are most probably amides (including amidic acids and their amides, and especially asparagin) and the carbohydrates.

3. The products of decomposition of proteids under the action of pure chemical agents. These are in the most part amides which are obtained by the action of bromine,* of barium hydrate under high pressure,† of hydrochloric acid and stannous chloride,‡ by long boiling with sulphuric acid,§ or acids in greater part from the fatty series, obtained through the action of manganese dioxide and sulphuric acid || as well as chromic acid.¶ Besides carbonic acid and oxalic acid are nearly always formed.

The presence of hippuric acid in the urine of herbivorous animals, the indol and the skatol found in the products of pancreatic digestion (Salkowski), the tyrosin nearly always present in the animal body, lead to the supposition that aromatic groups are also constituents of the proteid-molecule and it even seems that some of the most characteristic color reactions of these compounds are due to them.**

We shall not pass over in silence the synthesis of colloidal bodies obtained by Grimaux and by Schützenberger, which shows a very great resemblance to proteids. Grimaux obtained his colloid by melting together asparagic anhydride with carbamide; Schützenberger by the action of epichlorhydrin upon carbamide.

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* Hasiwetz and Habermann, Liebig's Annalen, 159 : 304.

† Schützenberger, Annales de Chimie et de Physique, V. 16 : 289.

‡ Hasiwetz and Habermann, Lieb. Ann. 169 : 150.

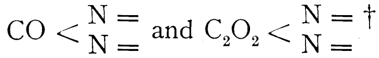
§ Kreusler, Zeitschrift für Chemie, 1870 : 93.

|| Guckelberger, Liebig's Annalen, 64 : 39.

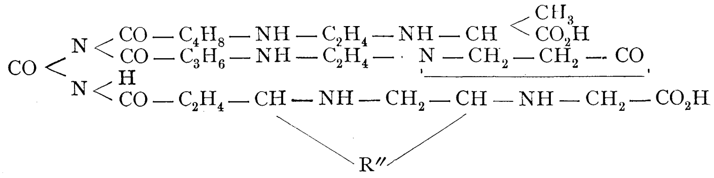
¶ *Ibidem.*

** So the reaction upon krautoprotein, by Millon and Liebermann. See Würtz. Second supplement au Dictionnaire de Chemie, 1892.

Taking into consideration the qualitative and quantitative relations of compounds derived from the proteids through the action of baryta-water under high temperature (200° C.) and pressure, Schützenberger[*] gives formulae for albumin and gelatin, which are perhaps premature as to minute details, but no doubt give quite an adequate expression of the fundamental facts both physiological and chemical by putting at the base of the proteid molecule, two groups, the carbamide and the oxamide :



$$CO < \begin{matrix} N = \\ N = \end{matrix} \text{ and } C_2O_2 < \begin{matrix} N = \\ N = \end{matrix}$$[†]



The presence of these two groups will enable us to give an account of two very general facts concerning the chemistry of organization.

This is practically all that we know about the final product of synthesis o nitrogen compounds in plants. We shall take next into consideration the physiological facts elucidating that synthesis.

In the first place may be noted the changes of proteids in the germination of seeds. As colloids, they cannot be transferred from cell to cell unless they are decomposed into simpler crystalline bodies. A long series of investigations has shown that this transfer is performed in the shape of amides.

Asparagin is to be found in very many sprouting plants while it is not present in their seeds. Asparagin is not soluble in alcohol and when sections of plants are placed in that liquid it is deposited in characteristic crystals. By means of this reaction Pfeffer has shown that many seedlings contain asparagin, whether growing in light or in darkness ; in other plants it is not present. This asparagin is obviously derived from the proteids contained in the seed and is used to build up the protoplasm of growing parts, as shown by the experiments of Boussingault and recently it has been confirmed by Laskowski, Fleury and Detmer, that when a seedling does not receive nitrogen from outside, the quantity of that element is not changed during the germination. In other words the plant does not lose its nitrogen.

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[*] See the article of that author in the second supplement to the Dictionnaire de Chemie of Würtz (1892).

[†] We quote here the condensed formula of Schützenberger, given by Goutier in his Chimie de la cellule vivantem, 1895 :

In the same conditions as sprouting seeds are the young buds of plants ; their tissues are formed from the plastic materials gathered by the plant during the foregoing summer. Thus Borodin found asparagin and sometimes tyrosin in the buds of many trees and shrubs, especially in those which are developed from twigs cut in winter and grown in dull light, indicating that light impedes directly the formation of asparagin : but Pfeffer obtained the same result when cultivating lupine in light, but in an atmosphere free from carbonic acid. Such plants were very rich in asparagin.

There is, moreover, no doubt that the accumulation of asparagin is connected with another fact, the lack of carbohydrates. In the described experiments this was due either to the cutting off of a twig (freed of leaves and thus unable to assimilate) from a tree, containing reserve carbohydrates, or to the suspension of assimilation through darkness or lack of carbonic acid. In plants, which are growing in normal conditions, the asparagin does not accumulate, for it is at once combined with the produced carbohydrates to form proteids. This inference is confirmed by the investigations of Schultze and Uhrich,[*] who found that the large part (34–47.7%) of nitrogen in the roots of the beet (*Beta vulgaris*) is contained in amides (specially in glutamin and asparagin), while during the growth of the leaves a large portion of these bodies is consumed to form the proteids for these organs.

Emmerling gives as the result of the first of a series of investigations, which he undertook in this line on a large scale, the following statement : "The parts of plants growing energetically contain more amide than parts which are older and already developed." [†]

At last experiments with seeds growing in darkness complete the preceding tests, showing that in this case amides and carbohydrates are produced from proteids. In the experiments of Uhrich, Schultz and Umlauf with seeds of lupine the result was that the plants cultivated in darkness contained 18.22% more of asparagin and 12.31% more of other amides and organic nitrogen compounds, while only 5.33% more of carbohydrates in form of glucose and cellulose than seeds ; on the other hand they contained 30.07% less of proteids. The comparatively slight increase of carbohydrates is explained by their loss from respiration, the general loss being 18.30% of dry substance.*

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[*] Landwirtschaftliche Versuchsstationen, **18** : (1875) and **20** : (1877).

[†] Landw. Versuchsstationen, **24** : 153. 1880.

All these facts prove without doubt that amides can be produced in plants from proteids, and can, together with carbohydrates, be used in their reconstruction ; they do not of course prove that we can consider amides as the predecessors of proteids when formed from inorganic substances, and the difficulty of the investigation in this line is based upon the fact that amides are always found in growing plants as the form in which proteids are transported either to the young growing parts (as buds, flowers, fruits) or to places where they are deposited as reserve food for the next year (roots, bulbs, etc.).

In order to answer this question A. Emmerling† undertook a long series of investigations, consisting of the quantitative determination of different compounds of nitrogen in different parts of plants and in various stages of their growth. These investigations led him to the belief that amides are products, preceding the formation of proteids from inorganic substances in plants.

Before this series was completed (1887) other pieces of work appeared which tend to show the same results.

Kellner was the first who tried to ascertain that proteids are formed from amides. He compared the amount of amides in plants supplied with pure water and those supplied with a solution of potassic nitrate.‡ His results were criticised by E. Schultze § chiefly on the ground that we cannot consider amides as products of the synthesis of inorganic substances, until we have proved that they are not produced from decomposition of proteids. In the meantime Hornberger and Raumer * applied the method of determination of proteids newly introduced by Stuzer. This method enables us to determine the amount of these bodies from other nitrogen compounds in plants: From the distribution of proteids and other nitrogen compounds in Indian corn, Hornberger and Raumer deduced that the amides in plants are not only derivatives of proteids, but also their predecessors produced synthetically.

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* Landwirtsch. Jahrbücher, **5** : (1876). The loss of dry substance is at the expense of fat (5.61%) and dextrine-like carbohydrates (10.02%), besides some other not fully explained substances.

† Landw. Versuchsstationen, **24** : 153 and foll. (1880).

‡ Landwirtschaftliche Jahrbücher, **8** : Suppl. 243. 1879.

§ Landw. Jahrbücher, **9** : (1880).

This result was confirmed by Hornberger,† who experimented upon *Sinapis alba* and by Emmerling who had then completed his long series of experiments.‡

On the other hand a recent publication gives a support for the second part of the supposition concerning the amides as a link between inorganic bodies and proteids. Barthold Hansteen cultivated *Lemna* in solutions of asparagin and glucose in darkness and found an obvious increase of proteids. The same result was obtained with carbamide and cane sugar as well as with salts of ammonia (ammonium chloride and sulphate) while leucin, cretin and alanin showed themselves unable to serve as proteid-producing materials.§ The last observation concerning salts of ammonium if proved to be true cannot be generalized, for experiments made under the most stringent conditions have proved that phanerogamic plants cannot use ammoniacal salts as a source of nitrogen, although that capacity is possessed by fungi.

So regarding the metamorphsis of nitrogen in plants we know that by the oxidation of the nitrates of the soil it is transformed into reduced nitrogen of amides, which by combination with carbohydrates produce proteids.

We, therefore, have to answer two questions :

1. In what organs of the plant does this transformation or its particular phases take place ?

2. What compounds are intermediate between nitric acid and

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* Landw. Jahrbücher, 11 : (1882).

† Landw. Versuchsstationen, 32 : 415. 1885.

‡ Landw. Versuchsstationen, 34 : (1887).

§ Beiträge sur Kenntniss der Eiweissbildung in phanerogamen Pflanzen. Berichte deutsch. bot. Ges. 14 : 362. 1896 the amides on the one hand, and between the latter and proteids on the other ?

The first can be answered with greater certainty owing to the investigation of the past few years. As to the second—it can be answered only hypothetically; but as every experimental science is in the same degree dependent upon facts, and upon scientific ideas, and as observation or experiment is always guided by scientific hypothesis, we ought not to throw aside suppositions which can be induced from facts and are not at variance with the general spirit of scientific thought.

As to organs in which proteids are produced a supposition was given as long ago as 1862 by Sachs,* who considered the leaves as active in this process, Hanstein was led to the same results by his experiments in girdling shoots, and Pfeffer showed that the development of blossoms and buds is dependent upon the presence of leaves and the supply of the substance which circulates in the sieve tubes.

The same conclusion must be drawn from the above quoted investigations concerning the distribution of nitrogen compounds in plants. The general result is that the organic nitrogen compounds are accumulated in leaves until the latter reach their full development, then they decrease in order to be transferred into fruits and finally disappear from the stem and the leaves while they are still increasing in the fruits even if the plant is not supplied with nitrogen from outside.

But quite decisive are the investigations of A. F. W. Schimper,† who showed by means of the reaction with diphenylamin that the nitrates pass as such through the fibro-vascular bundle of leaves and disappear in the chlorophylic cells of the mesophyl while large amounts of calcium oxalate is produced, the calcium being combined with the nitric acid as calcium nitrate entering the plant through the roots. This disappearance occurs only in light, while in darkness or in leaves free from chlorophyl the nitrates are accumulated in large amount. The experiments of Schimper and many others make it probable that in the same way sulphites and phosphites are decomposed in the leaves while the sulphur and phosphorus are used for building up the proteid molecules.

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* Botanische Zeitung, and the articles scattered through the 45 vols. of Flora (1862 *seq.*) where the cribrose vessels are indicated as the place of formation of proteids.

† Bot. Zeitung, 1888, N. N. 5–10.

We cannot however agree with the conclusion of the author that the chlorophyl grain is the organ of this synthesis, and that the last is produced only under the influence of light. The synthesis of proteids from sugar and nitrates or salts of ammonia in fungi deprived of chlorophyl contradicts such a supposition. This objection cannot be overthrown by the single remark, that the assimilation of nitrogen is in some regard different from this process in higher plants, for we have no reason to admit the necessity of light and chlorophyl to the production of proteids, since we know that such a synthesis can be produced without their influence. Of course the principal difference between fungi and chlorophyl-bearing plants does not consist in the fact that they produce proteids in different ways, but that one of the compounds necessary to that production (the carbohydrates) is taken from the outside.*

More probable is the supposition, that to the reduction of nitrates into amides or to the consequent transformation of amides into proteids some compounds or groups of atoms are necessary which are produced during the assimilation of carbon and constitute perhaps some stage in the synthesis of carbohydrates. In fungi such bodies could be produced by a retrogressive metamorphosis from carbohydrates, and the energy developed by this reaction may be used for the process of synthesis of proteids. Such a supposition is supported by the fact observed by the same author viz. : the presence of some reducing substance in the chlorophyl-bearing cells which hindered in many cases the color reaction with the diphenylamin, while neither the glucose nor the starch produce such effect.†

In this connection may be mentioned the hypothesis of Löw advanced long before the publication of the work of Schimper,‡ and based upon facts concerning the feeding of fungi. By studying experiments (especially those of Pasteur and Nägeli) to determine whether compounds with or without nitrogen can feed fungi Löw found that only the compounds containing the group CH.OH are able to produce proteids.

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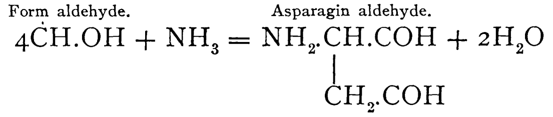
* Since this was written two pieces of work have appeared which have overthrown by experience the above exposed view of Schimper, previously advanced by Osc. Müller (Landw. Versuchsst., 1887). The one is of Kinoshita (Bulletin of Agricultural College in Tokio, 1895 : 2), the other the above quoted memoir of B. Hansteen (Berichte deutsche bot. Ges. 1896).

† l. c., 145.

‡ Eine Hypothese über die Bildung des Albumins in Pfüger's Archiv, **22** : 503. 1880.

He supposes then, that this reaction is performed in three phases :

The first consists in the production of asparagin aldehyde from the form aldehyde



$$\overset{\text{Form aldehyde.}}{4CH.OH} + NH_3 = \overset{\text{Asparagin aldehyde.}}{\begin{array}{l} NH_2.CH.COH \\ \quad\; | \\ \quad\; CH_2.COH \end{array}} + 2H_2O$$

The second is the condensation of several molecules of asparagin aldehyde into a body now unknown.

$$3\left\{\begin{array}{r} H_2N.CH.COH \\ CH_2COH \end{array}\right\} = \overset{\text{The unknown body.}}{C_{12}H_{17}N_3O_4} + 2H_2O$$

Löw maintains that this body is produced by the destruction of two or four aldehydic groups (COH).

At last the body thus obtained gives protein by the action of hydrogen and H_2S derived from decomposed water and reduced sulphuric acid :

$$6\,C_{12}H_{17}N_3O_4 + 6\,H_2 + H_2S = \overset{\text{The simplest formula of protein.}}{C_{72}H_{112}N_{18}SO_{22}} + H_2O.$$

This hypothesis was strongly criticised by E. Schultz.* Among his objections one deserves special notice, namely, that in scientific explanation such indefinite factors as the "vibrations of living molecules of protein" must be carefully avoided—a factor to which Löw has taken recourse again in his new hypothesis, concerning the formation of sugar.†

It would be useless to go into detailed criticism of this hypothesis ; but concerning the first reaction it may be remarked that while the given construction could be applied to the fungi, which can assimilate ammonia as easily as other nitrogen compounds, it could not apply to green plants, or at least to the phanerogams, since we know that they receive not only their nitrogen in the form of nitrates, but we have also certain data indicating the nature of the first transformation of these nitrates. And this is the only point in the whole process on which experimental researches have thrown some light.

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* Ueber die Eiweissumsatz in Pflanzenorganismus—Landwirtschaftliche Jahrbücher, 1885 ; Just's Berichte, 1888. The hypothesis proposed by this author to supply the place of that of Löw seems to me, indeed, more remote from probability than the criticised one.

† Berichte deutschen chem. Gesellsch., 22 : 482.

The purely chemical investigation of A. Emmerling* has shown that oxalic acid can decompose very dilute solutions of potassium or calcium nitrate by combining with the base and setting free the nitric acid.† The most interesting consequence of this fact is the behavior of the oxalic acid in the presence of nitrate of potassium and carbonate of calcium. Pure oxalic acid does not dissolve calcium carbonate, because of the formation of thin insoluble layers of calcium oxalate, which preserves it from the action of the acid. But as soon as we add a small quantity of nitrate of potassium it is decomposed, and the nitric acid, set free, dissolves the calcium oxalate, producing calcium nitrate, which is again decomposed by the prevailing mass of oxalic acid. Thus a small quantity of potassium nitrate can by its ferment-like action assist in dissolving a large amount of calcium carbonate.

Thus the observation of Schimper concerning the disappearance of nitrates in the green cells of leaves and the simultaneous accumulation of crystals of calcium oxalate, lead to the conclusion that the first change to which nitrates are exposed in leaves consists in setting free the nitric acid.

The nitric acid ought then to be the starting point of our construction. Such a reaction is presented in the hypothetical equation of A. Mayer, which represents this reaction :

$$\overset{\text{A carbohydrate.}}{C_{37}H_{74}O_{37}} + 6HNO_3 = \overset{\text{Protein without sulphur.}}{2C_{12}H_{19}N_3O_4} + 21H_2O + 13CO_2$$

The first member of this equation is a multiple of CH_2O—the simplest formula of carbohydrates ; the first number of the right side is the simplest formula of protein, with the omission of the sulphur.

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* Landwirtsch. Versuchsstationen, 34 : 109.

†R. A. Wood has proved the same for pure water (Amer. Chem. Journal, 1895).

The equation shows that nitric acid and a carbohydrate can produce protein without setting free other products than those which are produced by respiration, namely, carbonic acid and water.

This reaction is otherwise represented by Berthelot and André, whose considerations are based upon chemical researches concerning the amount of calcium carbonate, oxalic acid and proteids at different periods of life of a plant and in its different parts.*

Comparing the formula of formic aldehyde with that of oxalic acid, we see that when the latter is produced from the former it would have an excess of hydrogen :

$$2CH_2O + 2H_2O = C_2H_2O_4 + H_6$$

If we take into consideration the equality of volumes of carbonic anhydride and oxygen by the assimilation, the surplus of hydrogen shows that besides the oxalic acid a substance richer in hydrogen (or poorer in oxygen) than carbohydrates is produced. This may be the protein. The amount of protein found in the leaves of *Rumex acetosa* by the quoted authors answered fairly well to that of the oxalic acid, as needed by such a supposition. The oxalic acid is to be considered as one of the products oxydized, being formed as the reverse side of the reduction of proteids.

A. F. W. Schimper,† agreeing with Emmerling, that amides are the predecessors of proteids, considers in accordance with the expressed view of Berthelot and André the oxalic acid as an indirect product of that process and gives the following equation to explain the formation of amides :

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Nitric acid.}}{2NHO_3} = \underset{\text{Asparagine.}}{C_4H_8N_2O_3} + \underset{\text{Oxalic acid.}}{C_2H_2O_4} + 2H_2O + 3O$$

The three atoms of oxygen are added to the exhaled mass of that element giving a little surplus over the amount of absorbed CO_2, which is generally observed.‡

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*Comptes Rendus, 101 : 24, and 102 : 995, 1043.

† Flora, 1890. (Zur Frage der Assimilation der Mineralsaltzen in der Pflanze, 242.)

‡ *L. c.*, 260.

The above quoted equation is in accord with the views advanced by Holzner,* upon the participation of the oxalic acid, who considers it as a product of proteids, destined to decompose the nitrates, phosphates and sulphates of calcium introduced into the plant and to eliminate that element in an insoluble compound, a view which was confirmed experimentally as to the latter part by Emmerling. The quantitative relation of both oxalic and nitric acids is just the same in the above mentioned equation as needed by the supposition. It is two molecules of HNO_3 for one molecule of oxalic acid.

On the contrary Palladin† claims, that the oxalic acid is produced in the second stage of the reaction by the transition from amides into proteids and represents the process thus :

$$\overset{\text{Asparagin.}}{9\ C_4H_8N_2O_3} + \overset{\text{Glucose.}}{9\ C_6H_{12}O_6} = \overset{\text{Protein (without S).}}{C_{72}H_{112}N_{18}O_{22}} + 9\ C_2H_2O_4 + 23\ H_2O + 2H_2$$

This supposition does not contradict that accepted by Schimper's formulae ; Berthelot and André found that the amount of oxalic acid in the form of soluble oxalates in fresh leaves is nearly equal to that found with calcium thus agreeing with the last equation in which, as well as in that of Schimper one molecule of oxalic acid is produced for two atoms of nitrogen, the amount of oxalic acid combined with calcium (supposing that the nitric acid is absorbed by the plant only as calcium nitrate) being thus equal to its surplus over that element.

Such are the chief facts and the attempts to give an account of them. Trying to go somewhat further in this line the best way seems to me to start (as did Pfeffer) with a per cent. composition of both asparagin and a proteid, for instance legumin, calculated as to the equal amount of nitrogen, and to compare their difference with the percentage composition of a carbohydrate, as, *e. g.*, glucose. We have then the following table of which the first three columns are taken from Pfeffer's memoir ‡ the fourth is obtained by multiplying the percentage composition of the glucose with 0.91 in order to reduce its amount of carbon to the same number as represented in the third column :

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* Flora, 1867.

† Berichte deutsche bot. Ges. **5** : 326.

‡ Pringsheim's Jahrbücher, **8** : 355 *seq.* 1872.

Asparagin.	Legumin.	Difference.	Glucose.
C = 64.9	C = 36.4	+ 28.5	C = 28.5
H = 8.8	H = 6.1	+ 2.7	H = 4.7
N = 21.2	N = 21.2		
O = 30.6	O = 36.4	− 5.8	O 37.8
125.5	100.0		71.0

The difference between the two latter columns is

$$H = 2 = H_2$$
$$O = 43.6 \text{ or nearly } 2\tfrac{2}{3}\ O$$

which give together H_2O and about $1\tfrac{2}{3}$ O in surplus for each two atoms of N. In other words the transition from amides to proteids, supposing that the lacking elements are supplied by the carbohydrates, is a reduction. It may be, that this part of the synthesis requires the action of those strongly reducing bodies, which were found in the leaves by Schimper; for, as we know, the amides can be accumulated in darkness while the formation of proteids from them is connected with the action of light upon chlorophyll as stated by Schimper. The new experiments in this line* show indeed, that if the plants have at their disposition an abundant supply of carbohydrates they can produce this synthesis in darkness : This does not appear strange, for they are then placed in the same conditions which exist generally in fungi, the needed reducing energy being supplied by the oxidation of the surplus of sugar.

Another point to be considered is the origin of the oxalic acid. We have seen that Schimper as well as Berthelot and André consider it as an indirect product of the synthesis of proteids and this opinion generally prevails among physiologists in opposition to the older supposition of Holzner, who considered it as the result of their decomposition. On the other hand we see that oxalic acid is produced at nearly every decomposition or splitting of the proteid molecule.

The only fact which can throw any light about the question as to the true origin of oxalic acid in plants, is the distribution of that compound in different organs of the plant. This point, as regards the oxalic acid bound to the calcium and forming insoluble crystals, was very carefully studied by Schimper * and by Kohl.†

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*The above quoted pieces of work of Berthold, Hansteen and Kinoshita; W. Zaleski in Ber. deutsch. bot. Ges. 15 : 536.

The former distinguishes four types of calcium oxalates, adding a new one to the three proposed by Schimper : (1) The *primaries* are found in buds and are produced independently of assimilation ; (2) The *secondaries* originate in the chlorophylic parenchyma and are connected with assimilation ; (3) The *tertiaries* are found in the neighborhood of sclerenchymatous cells and also in fruits and seeds, where reserve-substances are accumulated ; (4) The *quaternaries* are deposited in leaves in the autumn and remain there.

Beginning now with the fourth category, we know, that in the autumn new proteids are not formed in leaves, but on the contrary, the larger portion of those that constitute the protoplasm of the cells, is dissolved and transferred into the stem ; this transfer is most probably accomplished after the splitting of the proteids into amides and carbohydrates. The most abundant production of oxalic acid in plants is then due to the decomposition of proteids. It can be objected that the larger part of oxalic acid could have been produced at any earlier period of development of leaves being then in form of soluble compounds and that the sudden appearance of a large amount of crystals in autumn is due to the supply of calcium. But we have no reason for supposing that large amounts of calcium are brought into the leaves at a time when their vital functions are declining.

In reality the above quoted results of Berthelot and André concerning the distribution of oxalic acid in *Rumex acetosa*, containing a large amount of soluble oxalates seems to contradict our conclusion ; but the same authors found in other plants (*Amarantus caudatus*, *Chenopodium quinoa*) quite opposite relations, and they add, after having presented the results of analysis of the first of these plants : "that these latter plants show quite a different mode of generation and physiological functions" (Ceci accuse un mode de génération et des fonctions physiologiques toute differentes†). It is then not improbable that such plants as *Rumex* and other "oxalic" plants represent some deviation from normal conditions.

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* The above quoted article in Bot. Zeitung, 1888.

† Anatomisch-physiologische Untersuchungen über Kalksaltze und Kieselsäure in den Pflanzen, 1889.

The deposition of calcium oxalate in places where thick membranes are formed was noticed long since by Sachs, and it is very natural to associate this phenomena with the production of cellulose. The only known method by which this body can be obtained is through the action of living protoplasm and that suggests the idea, that the cellulose is a product of proteids. A direct transition of other carbohydrates, as sugar or starch, into cellulose was never observed either in the chemical laboratory or in plants ; and we can not imagine the rôle of the protoplasm in this process, otherwise than by supposing that these carbohydrates become constituent factors in the proteid molecule, and from the reduction of this molecule the cellulose is derived.

Now the formation of the crystals of oxalates depends upon two factors : the formation of oxalic acid and the supply of calcium in amount sufficient to bind that acid. Both factors are present in the considered case, for where large amounts of cellulose are deposited by the protoplasm of the cells (as by the formation of sclerenchymatous elements), large supplies of carbohydrates are necessary to form anew the protein molecules of the protoplasm, and the transfer of carbohydrates, as both Schimper and Kohl have shown, is strictly connected with the presence of calcium, which is considered as a "vehicle" for these compounds.

In the same way we can explain the formation of oxalic crystals in buds ; the predominating phenomenon in this young tissue is the division of cells and the production of a large amount of cellulose membrane, which, according to our supposition, involves a large supply of calcium (as "vehicle" for carbohydrates) and the consequent formation of oxalic acid.

As to the oxalates (called secondary), formed in the chlorophylic tissue of the leaves, the conditions are more complicated. Schimper has proved that their formation "is dependent upon light and chlorophyl, but not upon assimilation."† Their

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[*] Comptes Rendus, 102: 1044.

[†] Bot. Zeitung, 1888, 69.

amount does not diminish when the plant is put in a position in which it cannot assimilate, as, for instance, is the case when is in an atmosphere deprived of carbonic anhydride. Schimper found that in such conditions proteids cannot be produced by the plant ; thus the formation of these crystals cannot depend upon the formation of proteids. But although the formation of proteids is one of the essential functions of the leaf parenchyma, we know that another process is taking place in them, and that is respiration. Whether or not we accept the view that the respiration of plants is essentially based upon the spontaneous dissociation of proteid molecules (the intra-molecular respiration), we cannot overlook the analogy between the function of carbohydrates as respiratory material in animals and plants.

When the assimilation goes on normally, the cellulose deposited by the decomposition of the proteid molecules augments the mass of the plant, while the assimilated carbohydrates are used partially in the reconstruction of that molecule, partially burnt out, the same as in the animal organism a product of proteids—the fat—is stored up, when the animal is abundantly fed with carbohydrates. But, if the production of carbohydrates is not sufficient, the material produced by the proteids is burnt the same as fat is burnt in animal organism under the same conditions. The amount of proteids decomposed by respiration, and consequently that of the oxalates is nearly the same, whether assimilation takes place or not; only in the last case the growth of the plant is hindered, for the part of the protein molecule deprived of nitrogen, will be oxydized into H_2O and CO_2, while its part containing nitrogen will increase the amount of amides, which, as is known, is really observed in such conditions.

The dependence of the secondary oxalates on light and chlorophyl can be explained by the proteid influence of these factors upon the transpiration or, in other words, upon the ascension of water from the soil towards the leaves, and the introduction of salts of calcium in quantities necessary to bind the produced oxalic acid. This dependence of the secondary oxalates upon transpiration was directly proved by Schimper*; the independence of the primary ones from that function, proved by the same investigator, can be explained from the above statement, namely, that the necessary amount of calcium is never lacking in this case, because these organs being unable to produce the carbohydrates indispensable for their growth, these bodies must be transferred to the other parts of the plant, and that transfer is, as we know, fulfilled in form of a combination with calcium.

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* *Loc. cit.* 89.

We can thus consider a large part if not all the oxalic acid produced in plants* as a final product of decomposition of proteids in a certain degree analogous to the carbamide in animals. The analogy is increased by the fact that the oxalic acid is either ejected from plants in the fallen leaves, or reduced to a state of insolubility from which, in most cases, it is never brought forth. It is thus an excretion, comparable to the carbamide in animals. If these inductions are true, we can propose a very general question : *What is the reason for such a difference between terminal products of decomposition of the proteid molecule in animals and in plants* ?

We find a fact of equal generality which enables us to answer this question : that is, the reduction of nitric acid, common to all chlorophylic plants. We do not know exactly the stages through which this reduction goes, but it is not to be doubted that one of them must be nitrous acid (NHO_2). The salts of this acid are found in plants only in very small amounts, but that is easily explained by their quick transformation into other compounds.

The action of nitrous acid or its anhydride upon the compounds of an amidic type is very characteristic. When the reaction is violent the place of the amido group (NH_2) is taken by the hydroxyl group (HO), the nitrogen being set free ; in other circumstances all the nitrogen remains combined with the radical, producing a diazo compound, while the hydrogen of the amido group and a part of the hydrogen of the radical is combined with the oxygen of the nitrous acid.

These two modes of action of the nitrous acid are exemplified by the following equations :

If we take the oxamide (the amide of the oxalic acid) the result will be oxalic acid, nitrogen and water :

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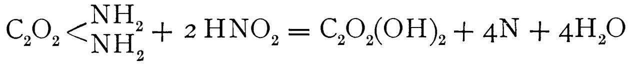
* Schimper admits that the whole amount of oxalic acid in plants need not necessarily have the same origin (*loc. cit.* 69).

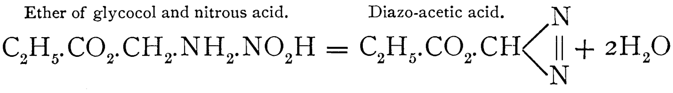
Oxamide. Oxalic acid.



$$C_2O_2<\begin{matrix}NH_2\\NH_2\end{matrix} + 2\,HNO_2 = C_2O_2(OH)_2 + 4N + 4H_2O$$

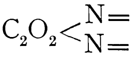
But, if we act cautiously with hyponitrous acid upon the glycocol (the amido-acetic acid) we obtain at first the ether of that acid and of the glycocol, which afterwards yielding water gives the diazo-acetic acid, as shown by the equation

Ether of glycocol and nitrous acid. Diazo-acetic acid.



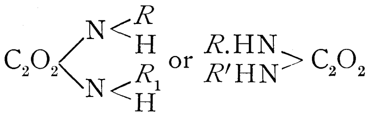
$$C_2H_5.CO_2.CH_2.NH_2.NO_2H = C_2H_5.CO_2.CH\left\langle\begin{matrix}N\\\|\\N\end{matrix}\right. + 2H_2O$$

We have seen that the chemical functions of the proteids compel us to admit the group



$$C_2O_2<\begin{matrix}N=\\N=\end{matrix}$$

as the basis of the molecule of these bodies. It is simply the oxamide group, introduced in one of the above given equations. Each of the two atoms of nitrogen has in that group two free units of affinity. If we denote with R and R_1 two univalent radicals containing C, H, O, N and S, we can represent a molecule of protein as follows:

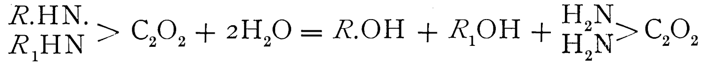


$$C_2O_2\left\langle\begin{matrix}N<\begin{matrix}R\\H\end{matrix}\\N<\begin{matrix}R_1\\H\end{matrix}\end{matrix}\right. \text{ or } \begin{matrix}R.HN\\R'HN\end{matrix}>C_2O_2$$

A molecule of such structure can give according, to the reagents which will act upon it, either carbamide or oxalic acid.

By hydrolysis it will be split at the places of junction of the radicals R and R_1 with the atoms of nitrogen, and the result will be as follows:

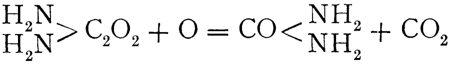
Protein. Oxamide.



$$\begin{matrix}R.HN.\\R_1HN\end{matrix}>C_2O_2 + 2H_2O = R.OH + R_1OH + \begin{matrix}H_2N\\H_2N\end{matrix}>C_2O_2$$

the oxamide then gives as known by oxydation carbonic anhydride and carbamide

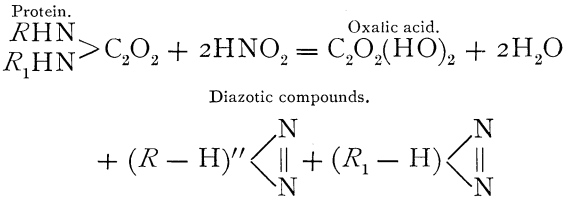
Oxamide. Carbamide.



$$\begin{matrix}H_2N\\H_2N\end{matrix}>C_2O_2 + O = CO<\begin{matrix}NH_2\\NH_2\end{matrix} + CO_2$$

The remainders of the first equation $R.OH$ and R_1OH are poorer in nitrogen than protein. It is the type of disaggregation of proteids in animals.

If the nitrous acid will act on such a molecule, it will split at the junction of the nitrogen atoms with the group C_2O_2, as was the case in the above quoted reaction with oxamide and nitrous acid; but if the reaction proceeds very slowly the nitrogen will not be set free. Keeping the analogy with the above given reaction of NHO_2 and the glycocol we can represent the supposed process as follows:



$$\overset{\text{Protein.}}{\begin{matrix}RHN\\R_1HN\end{matrix}}>C_2O_2 + 2HNO_2 = \overset{\text{Oxalic acid.}}{C_2O_2(HO)_2} + 2H_2O$$

$$\overset{\text{Diazotic compounds.}}{+ (R - H)''\left<\begin{matrix}N\\ \|\\ N\end{matrix}\right. + (R_1 - H)\left<\begin{matrix}N\\ \|\\ N\end{matrix}\right.}$$

In other words each of the radicals R and R_1 losing one atom of hydrogen (which is used for the production of water with the oxygen of the nitrous acid) and developing to a new value of affinity, combines with the now formed diazo group $(-N=N-)$.

This ought to represent the transformation of the proteids in plants, which, as we know, *do not lose their nitrogen.*

The known fact that the secondary and the tertiary amides of the aromatic series do not give diazotic compounds with nitrous acid,* cannot be quoted against the possibility of the supposed reaction. For (1) The diazotic compounds of the fat series differ much from those of the aromatic one as well in their constitution as in their properties, and (2) As we know very little about them the argument based upon the ignorance of such a reaction loses its strength. Then, we must remember that the reaction to which the production of diazotic compounds is due, belongs to those in which all is dependent upon the conditions of action of the agents; we are, indeed, encouraged to suppose that the subtleness of the transition in plants surpasses even the most delicate chemical operations in our laboratories that we can imagine.

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* From the secondaries nitroso-amides are obtained; the tertiaries give compounds with a nitroso group (NO) on the benzolic nucleus (Confr. Ladenburg, Handwörterbuch der Chemie, 3: 194).

The above presented hypothesis lacks direct proof. My own repeated attempt to produce the reaction of dissociation supposed for plants, either through action of gaseous N_2O_3 or nitrite of potassium (KNO_2), or soda ($NaNO_2$) in presence of any acid (hydrochloric or acetic) upon the albumin did not give any definite result.* This is certainly no objection against the validity of the hypothesis. The conditions for such a reaction are probably very delicate and very complicated and remain to be discovered. But among recent pieces of work there are some which give an indirect support to this hypothesis.

Drexel † obtained through action of hydrochloric acid and stannous chloride on albumin a base (*lysatine*, $C_6H_{13}N_3O_2$) which gives carbamide when treated with baryta water. This reaction shows, as the author deduces, that carbamide is not a product of oxydation, but of hydrolysis. The same result is reached through physiological deductions.‡ A fact of greatest interest for physiologists is that the arginine ($C_6H_{14}N_4O_2$ obtained by Schultze from the seeds of *Lupinus* and differing from lysatine only by the addition of NH, gives also carbamide with baryta water.§ It proves that a wide difference in the constitution of animal and vegetable proteins is not the ground for the different terminal products, but only the difference of the chemical processes in both.

On the other hand Buchner and Curtius, using soda nitrite on the product of the reaction of hydrochloric gas and alcohol on gelatine, obtained a diazotic compound, which differs very strikingly from other compounds of this kind by its stability; thus, *e. g.*, it can be boiled without decomposition. This compound was obtained in a large amount (150 grains from 400 grains of gelatine) and ought to be the only product of the reaction. The investigation of the products from the action of iodide upon these compounds leads the authors to one of the following formulae:

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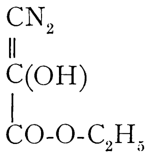
* In the first case it seemed not to be produced by any change in the albumin; in the second xanto protein was the constant product of the reaction.

† Ueber die Bildung des Harnstoff aus Eiweiss in the Berichte deutsch. chem. Gesellsch., 1890: 3096. Confr. also Siegfried, Zur Kenntniss der Spaltungsproducten der Eiweiss *Ibid*; **24**: 418. 1891.

‡ *Cf.* A. Gautier, Chimie de la cellule vivante, 1895, passim.

§ Ber. deutsch. chem. Ges. **24**: 1098. 1891.

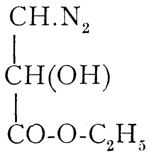
Ether of the diazo-oxy-acrylic acid.



$$\begin{array}{l} CN_2 \\ \| \\ C(OH) \\ | \\ CO\text{-}O\text{-}C_2H_5 \end{array}$$

or

Ether of the diazo-lactic acid.



$$\begin{array}{l} CH.N_2 \\ | \\ CH(OH) \\ | \\ CO\text{-}O\text{-}C_2H_5 \end{array}$$

These facts show the possibility of such a reaction, as was suggested both in animals and in plants which explain two very general physiological facts, viz. :

1. That the terminal products of disintegration of the proteid molecule are different in plants and in animals.

2. That green plants cannot use the ammoniacal compounds for the production of proteids, while the mediating links in that process are amides, compounds with hydrogenized nitrogen and not with the oxydized one as is found in nitric acid.

The first is explained by the fact that in the animal organisms there are no conditions for the production of nitrous acid, while in plants it might be produced as one of the stages of deoxidation of the nitric acid. The second, by the necessity of that acid for green plants and thus the impossibility of omitting that stage of deoxidation and of beginning the process with compounds containing only hydrogenized nitrogen. (In fungi, as we know, the process must be different, and it is proved that they can use the amidic ammoniacal compounds as supply of nitrogen.)

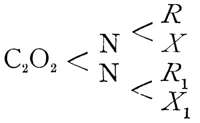
The logic of chemistry seems to impose upon us this hypothesis with almost irresistible necessity. All that we know about the chemical process in organisms teaches us that these processes are produced so as to pass through all the consecutive stages and compounds mediating between the initial and the terminal one. If we represent the transition from nitric acid to the amides through all known stages of reduction we obtain the following series of groups :

Nitro.	Nitroso.	Diazotic.	Hydrazines.	Ammonia and amides.
$-NO_2$	$-NO$	$-N=N-$	H_2N-NH_2	NH_3

We see thus that the diazo compounds take the middle place between the oxidized nitrogen of acids and the hydrogenized of hydrazines and amides. It is not improbable that the hydrazines are the agents of many reductions, such as the reduction of silver, which, as Löw and Bokorny have shown, constitute a property of living plasma, of those proteids in which the process of disaggregation and regeneration of molecules is still going on.

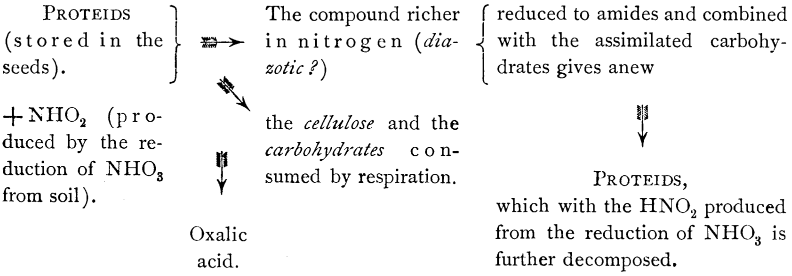
This regeneration is produced by means of compounds with diazotic groups like those formed in the last equation, and it is easy to see that every process of disintegration is accompanied by an enrichment with organic nitrogen, and as every decomposition of proteids is only a stage to their reconstruction in larger amount, the above discussed question upon the origin of oxalic acids appears under quite a new light; in most parts of the plant (excluding the autumnal leaves) both decomposition and reconstruction of proteids are connected processes taking place at the same time.

I have had already occasion to notice the analogy between the production of cellulose in plants and that of fats in animals. It is natural to suppose that both are produced by some groups of atoms set aside during the above discussed reaction. If in the formula of protein we put groups of atoms X and X_1 containing only carbon, hydrogen and oxygen, instead of NH, it assumes the following structure:



$$C_2O_2 < \begin{matrix} N < \begin{matrix} R \\ X \end{matrix} \\ N < \begin{matrix} R_1 \\ X_1 \end{matrix} \end{matrix}$$

These groups of atoms, being set free in each of the two supposed reactions, would originate the fatty acids in the case of animals, the cellulose or starch in the case of plants. Thus we can represent the continuous process of formation and destruction of proteids in plants as follows:



PROTEIDS (stored in the seeds).

+ NHO_2 (produced by the reduction of NHO_3 from soil).

The compound richer in nitrogen (*diazotic?*)

reduced to amides and combined with the assimilated carbohydrates gives anew

PROTEIDS, which with the HNO_2 produced from the reduction of NHO_3 is further decomposed.

the *cellulose* and the *carbohydrates* consumed by respiration.

Oxalic acid.

It is necessary to notice that this diagram does not require a constant relation between the amount of protein and that of carbohydrates in plants, a relation which really does not exist. The quantitative relation between compounds containing nitrogen and those without it in plants is within certain limits dependent upon the relation of assimilation to the amount of nitric acid absorbed from the soil. The greater the amount of assimilated carbohydrates, the less the carbohydrates, which are derived from proteids, will be burnt. On the contrary, when the amount of assimilated carbohydrates is not sufficient the relative amount of nitrogen in the plant will rise, and since in such conditions proteids cannot be regenerated—that surplus will consist of amides and other nitric compounds of a non-proteid character. Something like this we find in the above quoted results of the analysis of plants grown from seeds in darkness. (*Ante*, p. 39.)

But the amount of cellulose (and other soluble carbohydrates) cannot exceed a certain maximum in respect to nitrogen, and that maximum corresponds to such a state in which the assimilated carbohydrates make up all the loss for respiration, a state probably reached in plants when the conditions of feeding are normal.

Some chemical and physiological facts indicate that in the proteid molecule there are groups of atoms closely connected together and containing each 6 atoms of carbon. That number is basal for the carbohydrates found in organisms and fatty acids, the most common in animals, containing a multiple of that number (the stearic $C_{18}H_{36}O_2$; the oleic $C_{18}H_{34}O_2$). The amides, which are produced in organisms, or through the action of chemical agents upon the proteids contain generally less than 6 atoms of carbon and the most typical of them, leucine is the amido-capronic acid — $C_6H_{13}NO_2$. Only the less defined compounds obtained by Schützenberger (and called by him leucines) seem to make an exception to this rule, but we do not know whether these are chemical individuals or mixtures. All these and many other physiological facts oblige us to admit a near connection between proteids, fats and carbohydrates, and although the transitions from one group to another cannot yet be accomplished in the laboratory, there is no doubt that it is accomplished continually in the organisms themselves.

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